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A technical approach to dissecting and assessing cadaveric veins pertinent to chronic cerebrospinal venous insufficiency in multiple sclerosis

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Objective: To establish a detailed technical procedure for studying the anatomical correlates of chronic cerebrospinal venous insufficiency in cadavers of multiple sclerosis and control subjects, and to present our findings of the normal anatomic venous structures, with reference to previous descriptions from the literature.

Methods: This study examined the internal jugular veins (IJVs), the brachiocephalic veins, and the azygos vein from 20 cadavers (10 control and 10 multiple sclerosis). These veins were exposed, isolated by clamps from the rest of the venous system, flushed with water, and then injected with fluid silicone from the superior ends of both IJVs. After the silicone cured to its solid state, the venous tree was removed *en bloc* and dissected longitudinally to expose the luminal surface. All vein segments were analyzed for anatomic variation. Anatomical analysis for this manuscript focused on normal vein architecture and its variants.

Results: Thirty-seven of 40 IJVs contained valves: 29 bicuspid, 6 tricuspid, and 2 unicuspid. The average circumferences of the right and left IJVs were 2.2 and 1.8 cm, respectively. Thirteen of 20 azygos veins contained a valve, located on average 3.6 cm away from the superior vena cava junction. Nine of the 13 azygos valves were bicuspid; four were tricuspid. Only one of the 40 brachiocephalic veins contained a valve.

Discussion: We detailed a technical approach for harvesting cadaveric neck and thoracic veins with relevance to chronic cerebrospinal venous insufficiency. The anatomy of the venous system has significant variability, including differing number of valves in different regions and variable characteristics of the valves. Average vein circumference was less than that typically reported in imaging studies of live patients.

Keywords: CCSVI, Chronic cerebrospinal venous insufficiency, Multiple sclerosis, Extracranial veins, Internal jugular vein, Vein valves, Valves, Azygos

Introduction and Background

The extracranial venous drainage system returns blood from the central nervous system (CNS) back to the heart via several venous systems in the neck and along the spine. This venous system, in particular the internal jugular veins (IJVs) and the azygos veins, are of key importance in the chronic cerebrospinal venous insufficiency (CCSVI) hypothesis of multiple sclerosis (MS), which proposes that obstructions of venous drainage leads to the development of MS.[1] As a means of compensating for this venous obstruction, Zamboni *et al.* described the formation of 'substitute circles', which are collateral pathways for the blood to bypass the stenoses, thus preventing intracranial hypertension.[2] CCSVI is postulated to occur when these collateral pathways become overloaded. The resultant venous congestion and reflux into the CNS have been suggested as a possible contributor to the development of MS.

Structural abnormalities were found more commonly in the IJVs than the azygos veins.[1] Radiographic studies from some but not all groups have suggested that these abnormalities lead to obstruction in the venous drainage of blood from the CNS. Based on venographic imaging, several types of abnormalities were described to cause stenosis: annulus (circumferential stenosis of the venous wall), septum/valve malformation, intraluminal membrane obstruction, hypoplasia, agenesis,

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and twisting.[1] These structures have been classified as truncular venous malformations[3] and are thought to arise from defects in the embryologic development of the venous wall.[4] Based upon their location, some of the noted structural abnormalities could represent malformed venous valves.

Despite many radiographic descriptions of anatomical structures related to the CCSVI hypothesis, pathological and histological studies are lacking. Understanding the extracranial venous tree for structural abnormalities requires a thorough understanding of the normal vein and valve anatomy, including variations in the population.

The jugular veins exit the skull bilaterally through the jugular foramina. Near the level of the clavicle, each IJV joins with the subclavian vein to form the right and left brachiocephalic veins, which in turn join with each other to form the superior vena cava (SVC). The IJV lumen area gradually becomes greater in diameter as it descends toward the SVC. This gradual increase in diameter is interrupted at two sites, where the IJV lumen becomes slightly more dilated near its exit from the skull (known as the superior bulb) and just before joining with the subclavian vein (known as the inferior bulb). Along its path, the IJV receives several venous tributaries, including the occipital, pharyngeal, facial, lingual, and thyroid veins.[5] The inferior bulb is the most relevant segment of the IJV to the CCSVI hypothesis, because it contains the IJV valve and has been frequently cited as a location of abnormalities in CCSVI studies. The IJV valve is often referred to as the only valve between the heart and the brain.[6–8]

The azygos vein generally originates anterolateral to the L1 or L2 vertebra, from the junction of the right subcostal vein with the right ascending lumbar vein.[9] The exact origin of the azygos vein is variable.[10,11] The azygos vein continues superiorly through the posterior abdomen, anterolateral and adjacent to the vertebral bodies. After entering the thorax through the aortic hiatus or sometimes the right crus of the diaphragm, the azygos vein tends to the right side. As it approaches the T4 vertebra, the azygos arches anteriorly to form the azygos arch above the right main bronchus, and finally joins the SVC.[9,10] The azygos receives multiple smaller and variable tributaries along its path, including intercostal veins, the hemiazygos vein, the accessory hemiazygos vein, esophageal, mediastinal, pericardial, and right bronchial veins.

The main objective of this technical manuscript is to describe in detail the methodological aspects of the postmortem isolation and harvest of jugular and azygos venous systems. We then describe the anatomic variation in the architecture of normal jugular and azygos venous structures from 20 cadavers, providing reference to previous descriptions of these systems.

A comparison of pathological findings in MS versus control donors will be presented in a separate report.

Methods
Subjects
A total of 20 cadavers (10 with MS and 10 without MS) were dissected. There were 13 females and seven males with an average age of 61.7 years. All donors were obtained through one of two programs available to us: the Multiple Sclerosis Tissue Donation Program and the Cleveland Clinic Body Donation Program. The participants (pre-mortem) or next-of-kin (post-mortem) consented to the use of the bodies for research. The research program, including informed consents and protocols, were approved by the Cleveland Clinic Institutional Review Board.

General approach
The venous system was accessed through the anterior chest. The venous block containing the bilateral IJVs, subclavian veins, the SVC, and the azygos vein was isolated, flushed with water, and then injected with liquid silicone. After the silicone cured to its solid state, the veins were removed *en bloc* and dissected along the luminal surface for further examination. For this manuscript, we focused on the architecture and variation in normal venous structures that are to be expected when performing the dissection. Our analysis combines the control and MS subjects into the one pool of 20 subjects, since only the normal aspects of all veins are addressed.

Materials
Silicone reagents were obtained from Dow Corning, Midland, MI, and include silicone thinner (Xiameter® PMX-200), silicone rubber (3110 RTV base), and catalyst (Dow Corning® 4 Catalyst). Addition of powdered colored paint helped to facilitate visualization of the silicone cast. The silicone mixtures were adapted from Sanan *et al.*[12] A summary of additional supplies required for vein harvesting are listed in Table 1.

Technique for dissection
Dissection for access to the veins
A U-shaped incision was made through the skin and subcutaneous tissue extending from the acromion processes bilaterally to the third intercostal space. The resultant tissue flap was reflected superiorly toward the mandible to visualize the IJVs. Subcutaneous fat was dissected to expose a 6–8 cm segment of each IVJ at the level of the thyroid gland. Each subclavian vein was exposed by a 6–8 cm incision into the pectoralis major muscle just inferior and parallel to the mid-clavicle. Using Wheatlander retractors, the incision was opened further and carefully dissected down to the vein. The third and
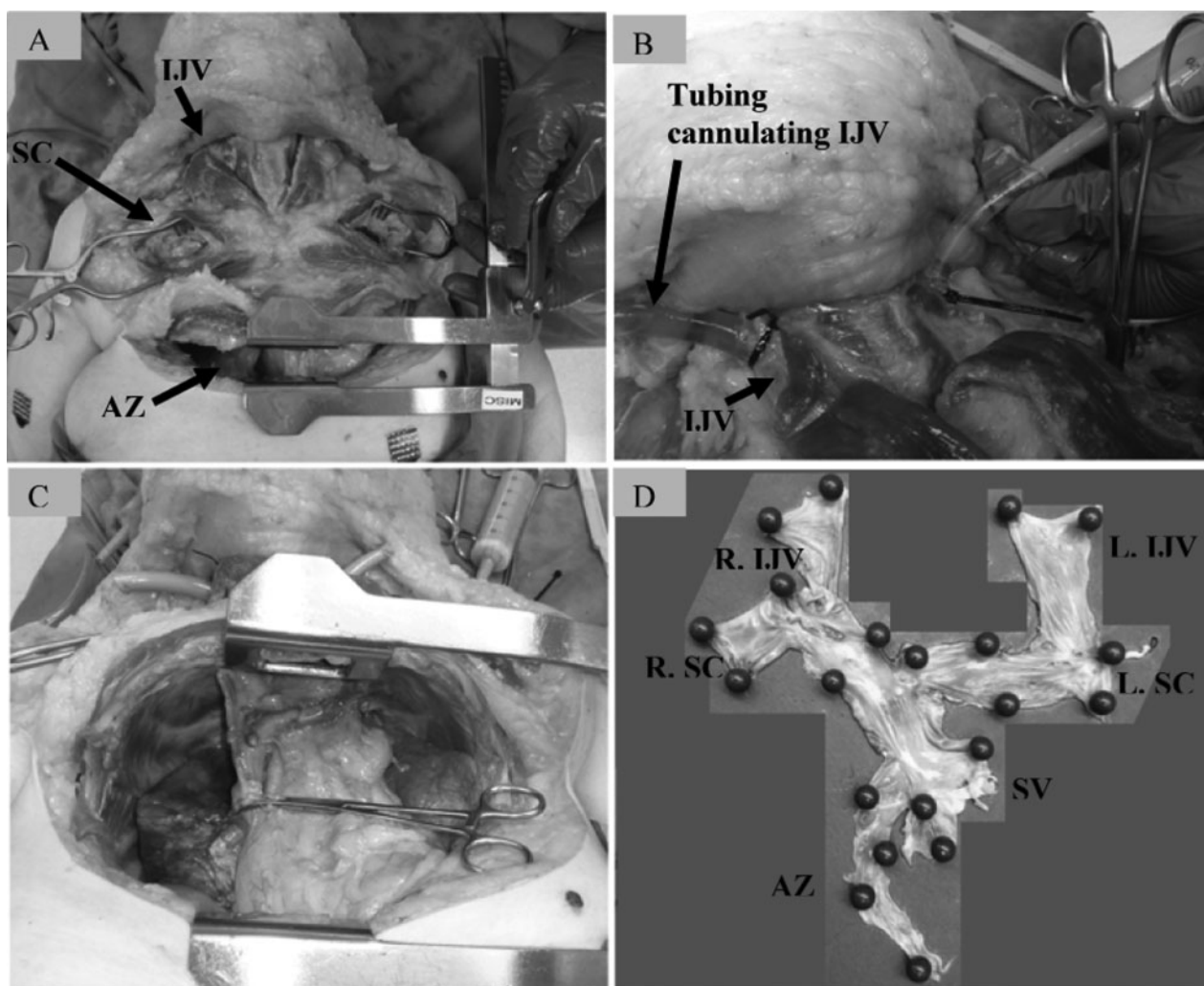
Figure 1 Dissection of internal jugular veins (IJVs) and azygos (AZY) veins. (A) Cadaver showing approach to exposure of IJVs, subclavian (SC) veins, superior vena cava (SVC), and AZY veins. (B) Cannulated IJVs after flushing with water. The left IJV is about to be perfused with silicone. (C) Extracranial venous system after silicone has been injected. (D) Dissected extracranial venous system pinned onto cork, with the luminal surface exposed for analysis of intraluminal structures.

fourth ribs were separated by incising the intercostal muscles. The sternum was transected using a bone saw. Figure 1A shows the dissection up to this point.

Table 1 Materials needed for cadaver dissection

Scalpel handle (No.3) and blade (No. 10)
Bone saw (for opening the chest cavity)
Chest retractor
Wheatlander retractors for exposure of subclavian veins
Plastic tubing (outer diameter: 7.9–12.7 mm)
Hemostats (5), forceps, scissors
Plastic cable ties
60-ml syringes (2)
Suction device
Teaspoon measure
125 ml screw cap specimen cup
250 ml or larger bowl
Tongue depressors or other instrument to mix silicone reagents
Corkboard
Push pins
10% neutral-buffered formalin
Container to store specimen
Ruler
Digital camera
Probes to demonstrate valves and other luminal structures

To access the IJVs, the sternocleidomastoid muscles were transected approximately 6 cm from their insertion onto the sternum and clavicles, and the cut ends were reflected superiorly and inferiorly.

Vein cannulation and transection
IJVs

The subcutaneous tissues were dissected away from IJVs to obtain visualization of, and circumferential access to, each IJV. Using a scalpel, an incision was made into each IJV that was large enough to accommodate the perfusion tubing. The tubing was then inserted into the lumen of each IJV and directed toward the heart. A cable tie was placed around each vessel wall to secure the tubing. Figure 1B shows the cannulated IJVs.

Subclavian vein

Each subclavian vein was dissected and clamped with hemostats. The veins were then transected lateral to the clamp. Clamping is necessary to direct the flow of injected silicone toward the azygos vein.

SVC

Soft tissues surrounding the SVC were dissected until the pericardium and SVC were visualized. Using a hemostat, the SVC was clamped where it enters the heart and then transected inferior to the clamp.

Azygos vein

The right lung was reflected toward the midline to expose the junction of the azygos vein with the SVC. The azygos vein was then transected where it enters the thoracic cavity. The azygos vein usually enters the thoracic cavity through the aortic hiatus, but it sometimes enters through the right crus of the diaphragm.

Perfusion

Using a 60-ml syringe, the venous tree was flushed by injecting each IJV with at least 120-ml of water. A suction device was used to remove the blood and water that accumulated in the thoracic cavity.

Preparation of silicone for injection

The timing and order in which the following steps are performed are important. One teaspoon (~ 5 cc) of paint powder was placed into a 125 ml screw-cap specimen cup; 50 ml of thinner (Xiameter® PMX-200) was also added to the cup, which was then covered and shook vigorously. Using a 60 ml syringe, 100 ml of 3110 RTV silicone was placed into a 250 ml or larger bowl. The powder paint/thinner solution was poured into the bowl with the silicone. This was then stirred until the solution became homogeneous. The next step should only be done when the veins are ready for injection because, once the catalyst is added to the silicone, it will start to cure within 10–15 minutes.

Catalyst-4 (20–23 drops) was added to the bowl containing the silicone and stirred well.

Using a new syringe, 60 ml of the silicone and catalyst mixture were drawn up.

Injection of the silicone

The 60 ml syringe containing the silicone/catalyst mixture was attached to the plastic tubing cannulating the left IJV (Fig. 1B). All of the silicone solution was then injected into the left IJV and the tubing clamped. The remaining 40 ml of silicone was drawn into the 60 ml syringe, injected into the right IJV, and the tubing clamped. Perfusion of the right IJV requires less silicone because the right brachiocephalic vein is shorter. The silicone then cured in 20–30 minutes. Covering the veins with warm moist towels will speed the curing process. Figure 1C shows the extracranial venous system containing the cured silicone.

Removal and fixation of veins

Once the silicone cured completely, the vessel walls were dissected to mobilize the IJVs, brachiocephalic veins, and azygos vein. The medial two-thirds of the clavicles were removed to help mobilize the IJV–subclavian junction. The entire venous tree was extracted in one block. Using a small scissors, the vein walls were transected longitudinally along the anterior or posterior plane of the venous tree to expose the luminal surface of the veins. Care was taken to not cut through any valve leaflets or other intraluminal structures. The silicone cast was removed and saved. The veins were pinned onto a corkboard with the exposed luminal surface upwards (Fig. 1D), and then placed into a container containing 10% neutral-buffered formalin for 12 hours or more.

Data documentation and analysis

A dissecting stereomicroscope equipped with a digital camera was used to visually inspect and document the anatomy of each sample. Inclusion of a ruler in each photomicrograph facilitated measurements of gross anatomical structures. The anatomy of valves and other membranous structures were highlighted in photographs by insertion of metal or wooden probes. In our experience, wooden probes dipped in ink and coated with paraffin worked best. The circumference of regions of interest from each venous segment was measured on the vein specimen itself. The silicone casts were stored to aid in the identification of valves or any other intraluminal structures.

Results

IJVs

Out of the 40 total IJVs examined in 20 cadavers, 37 IJVs contained semilunar valves immediately cephalad to the junction with the subclavian vein. No valve was present on the right side in two cadavers and on the left side in one; no cadaver lacked valves bilaterally. Out of the 37 IJV valves, 29 (78.4%) were bicuspid. These valves were composed of two thin leaflets that formed pocket-like structures facing opposite to each other along the vein wall (Fig. 2A). The opening of the pockets was always oriented toward the heart and never toward the brain. In general, the attachments of the valve leaflets were directly adjacent to each other, and the lengths of the edges of the cusps were similar. Tricuspid valves were observed in six of 37 IJVs (16.2%). One cadaver contained tricuspid valves in both the left and the right IJV. The three leaflets comprising tricuspid valves were similar in structure to those of the bicuspid valves (see Fig. 2B); however, a tricuspid valve did not necessarily consist of three symmetrical cusps. As an example, Fig. 2C depicts a tricuspid valve in which two of the cusps are relatively smaller than the third. Two unicuspid valves (5.4%) were observed in two cadavers. The single cusp also resembled a pocket-like structure, but the length of
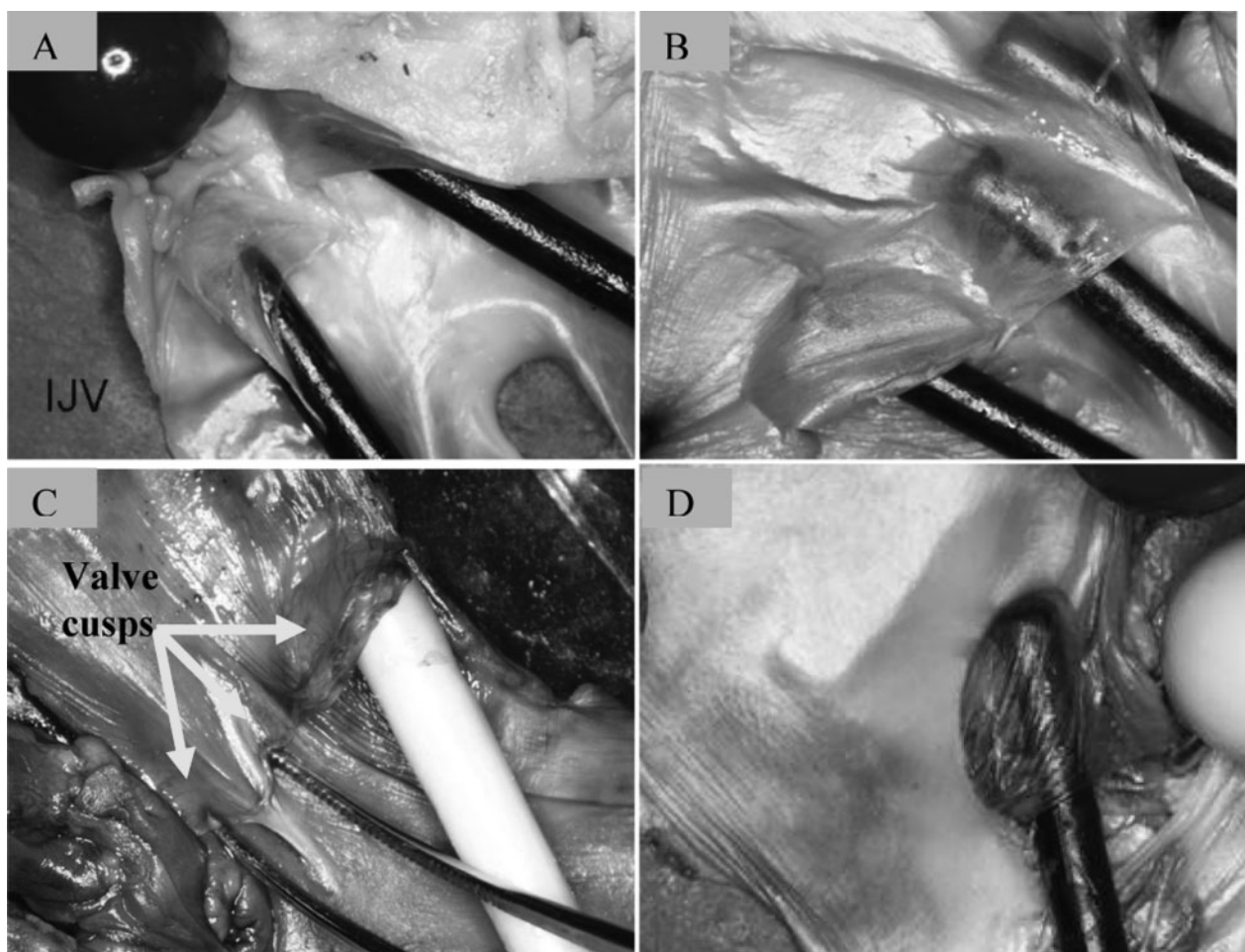
Figure 2 **(A) Normal bicuspid IJV valve. (B) Tricuspid IJV valve. (C) Asymmetrical tricuspid IJV valve. One leaflet (top) is normal in size and the other two are much smaller. (D) Unicuspid IJV valve.**

the edge was often small relative to the circumferential area of the vein wall (Fig. 2D). We postulate that valves with this morphology would be functionally insufficient. The average circumference of the right IJV at the level of the valve was 2.2 cm, with a range of 0.8–3.4 cm; that of the left IJV was 1.8 cm, with a range of 0.7–2.9 cm.

Azygos vein

A single azygos vein was present in all 20 cadavers examined. A valve was observed in 13 (65%) of the veins; nine were bicuspid and four were tricuspid. No unicuspid valves were present. The opening of the cusp pocket pointed toward the heart and never inferiorly. The valves were similar in structure to those of the IJV valve, but were generally smaller in size — proportionate to the smaller circumference of the azygos vein. Although some valves appeared to be insufficient (i.e. did not completely occlude the azygos when closed), most were well-formed in overall structure (Fig. 3A). The valve was located between 1.6 and 7.0 cm from the junction of the azygos with the SVC (mean = 3.6 cm). There was no preferential location of the valve along this continuum. No valves were ever observed at the junction of the azygos vein with the SVC. The average circumference of the azygos at its junction with the SVC was 1.8 (0.9–3.0) cm.

Brachiocephalic and subclavian veins

Out of the 40 brachiocephalic veins examined, 39 were valveless. One brachiocephalic vein on the left contained a unicuspid valve that, due to its very small dimensions, was most likely insufficient in preventing reflux. There was sufficient subclavian venous tissue for examination in 36 veins, and a valve near the junction with the IJV was observed in 35 of these. Compared to the IJV, the subclavian valve was more consistently bicuspid: 31 of 35 valves were bicuspid, 2 were unicuspid, and 1 was tricuspid. The structure of one subclavian valve could not be determined because it was damaged during the dissection.

Other venous branches

Numerous and variable smaller veins join the IJVs, subclavian, brachiocephalic, and azygos veins. These veins consistently had small bicuspid valves located just before their union with the larger vein. The sizes of these valves were proportionate to the lumen area of the branch. Figure 3B shows an example in which a collateral branch joins the azygos vein.
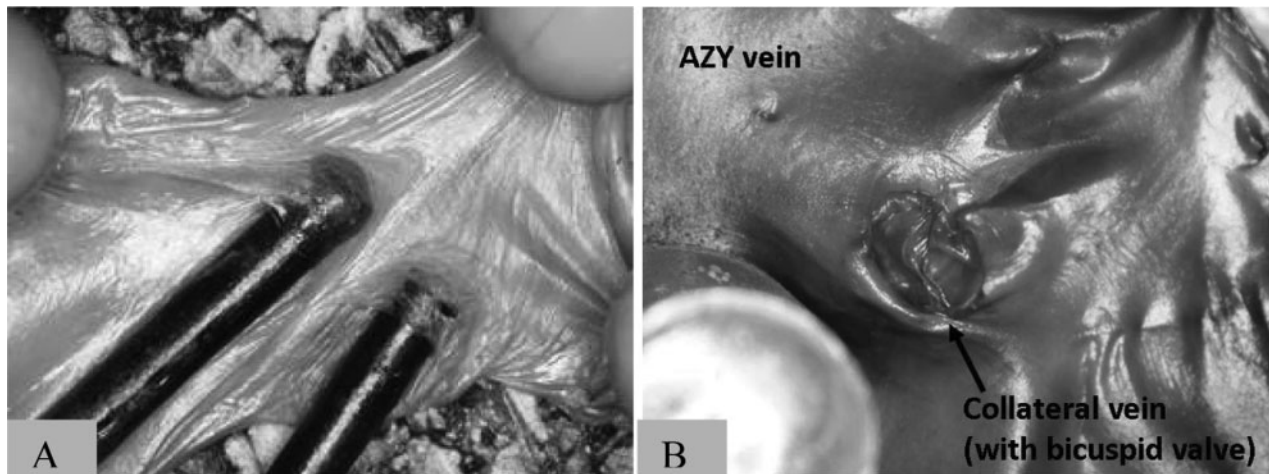
Figure 3 (A) Bicuspid azygos (AZY) valve. (B) Valve of a collateral branch entering azygos vein. The view is into the pockets of a closed semilunar valve.

Discussion

IJVs

The IJV anatomy has been described previously in both autopsy and imaging [computed tomography (CT) and ultrasound] studies (Tables 2–4). The consensus from multiple analyses, including ours, shows that the lumen of the right IJV is larger than the left (Table 2). We examined only the normal segments of the veins to determine the lumen size; circumference was never measured at points of focal stenosis. Our calculated IJV diameters were comparable to autopsy results from Dresser and McKinney.[6] As can be seen from Table 2, IJV lumen area is generally found to be larger on imaging compared to autopsy studies. This is not unexpected because the veins of live subjects

contain blood under pressure, and there is considerable compliance of the venous wall *in vivo*. For the purposes of CCSVI histopathological research, stenoses may be better defined as a percentage reduction in lumen area rather than by an absolute cutoff value. Tartiere *et al.* independently measured both the maximal diameter and the area of the IJV on CT-scan images.[13] These data demonstrated that approximating the IJV lumen as circular in cross-section would overestimate the lumen area. In fact, from our observations, the IJV lumen cross-section more closely resembles an ellipse but is often irregularly shaped. As already noted, the lumen area depends on the level at which it is measured due to the natural taper of the IJV and the presence of the inferior and superior bulbs.

Table 2 IJV lumen size

Reference	Study type	No. of subjects	Diameter R (mm)	Diameter L (mm)	Level of measurement
Our results	Autopsy	20	6.9*	5.7*	IJV valve
Dresser and McKinney (1987)[6]	Autopsy	7	7.3*	5.3*	IJV valve
Lim *et al.* (2006)[22]	CT	88	14.3	11.7	Cricoid cartilage
Tartiere *et al.* (2009)[13]	CT-diameter	190	17.0†	13.0†	Cricoid cartilage
Macchi and Catini (1994)[16]	Ultrasound	120	13.8		Ostial level

Notes: CT, computed tomography; IJV, internal jugular vein.
*The values shown are the calculated diameter, assuming a circular cross-section; only normal venous segments were assessed to determine lumen area.
†Tartiere *et al.* also measured the cross-sectional areas on CT images: right=16.0 mm²; left=10.2 mm².

Table 3 Prevalence of IJV valves

Reference	Study type	No. of subjects	% bilateral valves	% unilateral valves (right; left)	% absent valves
Our results	Autopsy	20*	85%	15% (5%; 10%)	0%
Dresser and McKinney (1987)[6]	Autopsy	7	100%	0%	0%
Silva *et al.* (2002)[8]	Autopsy	30	100%	0%	0%
Lepori *et al.* (1999)[15]	Autopsy	75	84%	9.3% (6.7%; 2.7%)	6.7%
Lepori *et al.* (1999)[15]	Ultrasound	75	60%	26.7% (21.3%; 5.3%)	13.3%
Akkawi *et al.* (2002)[7]	Ultrasound	125	85.6%	11.2% (9.6%; 1.6%)	3.2%
Macchi and Catini (1994)[16]	Ultrasound	120	71.7%	28.3%	0%

Notes: IJV, internal jugular vein.
*Our results may not be directly comparable to those of prior literature, since 10 of 20 subjects were diagnosed with multiple sclerosis. Other abnormal structures were not counted from either group (controls or multiple sclerosis).

IJV valves have been visualized in most patients examined and are located approximately 2–2.5 cm cephalad to the IJV–subclavian junction.[5,14] Autopsy studies revealed the presence of IJV valves in 93–100% of subjects (Table 3).[6,8,15] Similarly, we identified bilateral valves in 17 of the cadavers examined and unilateral IJV valves in the remaining three. Ultrasound studies generally reveal a greater percentage of subjects without IJV valves compared to autopsy studies, but this is most likely due to the increased difficulty of visualizing the valves with ultrasound.

The IJV valve architecture most commonly consists of two semilunar cusps (leaflets). The normal valve leaflets have been described as thin translucent structures that resist inversion.[6,8] On ultrasound, the valves can be observed as thin curvilinear echogenic structures.[15] In previous descriptions, only a minority of IJVs have been found to contain unicuspid or tricuspid valves (Table 4). Consistent with these reports, our results also showed that the IJV valve is most commonly bicuspid; tricuspid valves were the next most common. Unicuspid valves were rare. All three cusps of a tricuspid valve resembled the normal bicuspid valve leaflet structure. Hence, from a qualitative analysis, we speculate that most tricuspid valves would be properly functional and represent a normal variation of anatomy. Exceptions would include asymmetrical tricuspid valves, in which one or two of the cusps can be hypoplastic (Fig. 2C).

Results on valve function from different studies vary widely.[6–8,16] Ultrasound studies[7,16] report a higher prevalence of incompetence compared to autopsy studies,[6,8] but there is a lack of standardization for defining valvular incompetence among these studies.

The relationship between valve anatomy and function has been examined in the autopsy studies.[6,8] In both studies, unfixed specimens were connected to a manometer, fluid pumped in, and the pressure inside the lumen measured until the valve became incompetent. Both groups concluded that the tricuspid valves are competent when compared to the normal bicuspid valves. Two of the six tricuspid valves that we observed were found in the same subject, which has been reported previously.[6] The increased prevalence of tricuspid valves compared to unicuspid valves further supports that tricuspid valve architecture may be a normal variant. Silva *et al.*[8] showed that unicuspid valves were insufficient, allowing for retrograde flow. Although we did not functionally analyze any of the valves in our study, the gross anatomy we observed in the unicuspid valves suggests that these valves would have been incompetent (Fig. 2D).

Azygos vein

The valves of the azygos vein have not been as closely studied as the IJV valves. Although results of early imaging studies suggested that there were no valves in the azygos system, more recent CT-based studies describe the presence of azygos valves, which were all located in the azygos arch.[17–19] As previously reported, the clinical significance and functionality of the valves still remains unknown. Some of the valves may be incompletely formed.[17] Table 5 depicts the three CT-based imaging studies that found curvilinear structures resembling valve leaflets in the azygos arch; 53–68.2% of subjects whose azygos vein was visualized contained such structures. Yeh *et al.* reported the valve to be on average a distance of

Table 4 Subtypes of IJV valves

	Study type	No. of valves	% bicuspid	% tricuspid	% unicuspid
Our results	Autopsy	37*	78.4%	16.2%	5.4%
Dresser and McKinney (1987)[6]	Autopsy	14	71.4%	28.6%	0%
Silva *et al.* (2002)[8]	Autopsy	60	93%	2%	5%
Lepori *et al.* (1999)[15]	Autopsy	133	98.5%	0.8%	0.8%
Lepori *et al.* (1999)[15]	Ultrasound	110	38.2%		
Akkawi *et al.* (2002)[7]	Ultrasound	228	99.1%	0%	0.9%
Macchi and Catini (1994)[16]	Ultrasound	206	75%	10%	15%

Notes: IJV, internal jugular vein.
*Our results may not be directly comparable to those of prior literature, since 10 of 20 subjects were diagnosed with multiple sclerosis. Abnormal structures were not counted from either group.

Table 5 Prevalence of valves in the AZY arch

	Study type	No. of AZY veins	% with valves	Average distance from SVC junction
Our results	Autopsy	20*	65%	3.6 cm (1.6–7.0 cm)
Yeh *et al.* (2004)[17]	CT	154	68.2%	1.9 cm (0.5–4.0 cm)
Ichikawa *et al.* (2008)[19]	CT	194	53%	
Steinke and Moghaddam (2009)[18]	CT	191	64.9%	

Notes: AZY, azygos; CT, computed tomography; IJV, internal jugular vein; SVC, superior vena cava.
*Our results may not be directly comparable to those of prior literature, since 10 of 20 subjects were diagnosed with MS. Abnormal structures were not counted from either group.

1.9 cm away from the azygos junction with the SVC. Limitations of all three of these studies include an inability to clearly define the structure of the valve (bicuspid, tricuspid, abnormal intraluminal web or membrane, etc.) and the possibility of missing the presence of valves when no contrast enters the azygos vein from the SVC. Our autopsy findings confirmed the presence of such a valve; additionally, we were able to analyze its architecture. We found a valve in 65% of the azygos veins examined, comparable in frequency to that reported in the imaging studies. We found the distance of the valve into the azygos arch to be greater than Yeh's findings on CT scan (Table 5). Additionally, the exact location of the valve into the azygos vein was considerably variable. Nonetheless, it is important for imaging studies to not confuse the presence of an azygos valve with an abnormality. The consensus in the literature is that most azygos valves are insufficient. Based on our qualitative assessment of the valves, we believe that many azygos valves could be competent, when present. The valve cusps were well-formed and sometimes were similar in size to the IJV valve leaflets, especially in patients with smaller IJVs. Steinke and Moghaddam investigated the frequency of contrast material that bypassed the valve, which was interpreted as a surrogate for valve insufficiency: 53.2% of the azygos arch valves were found to be insufficient by this definition.[18]

Brachiocephalic and subclavian veins

Although not explicitly described in the CCSVI criteria, the brachiocephalic and subclavian veins have intimate connections with the IJVs and azygos veins. The subclavian veins join with the IJVs on each side to form the left and right brachiocephalic veins, which in turn merge to form the SVC. In one study of 97 cadavers, the subclavian valve was found in 91% of veins studied: it was bicuspid in 75%, unicuspid in 12%, and tricuspid in 4%.[20] Valves in the brachiocephalic veins have rarely been observed. In the same study of 97 cadavers, Anderhuber reported that most of the valves that were observed were unicuspid and located in the left brachiocephalic vein (which is longer than the right). The unicuspid valves appeared to be insufficient. Similarly, our results showed that the brachiocephalic veins appear to be practically valveless, as we found only one small unicuspid valve on the left side of one subject.

Conclusion

To date, the CCSVI hypothesis has only been evaluated by radiographic studies. Gross anatomical and histological analyses of the veins related to CCSVI are necessary for further assessment of the hypothesis. The main goal for this paper was to establish a methodology that can be used in the future for post-mortem analysis of venous abnormalities in the jugular and azygos venous systems. The anatomy of the venous system is generally much more variable compared to that of the arterial system. Therefore, a thorough understanding of the normal vein and valve anatomy is required before effectively evaluating venous specimens for abnormalities. A recent study from Dolic *et al.* showed an excess of intraluminal abnormalities in MS patients when compared to healthy controls, as visualized by ultrasound.[21] The vein lumen area we measured at post-mortem tended to be about half as large as the area measured from images (CT or ultrasound), which is likely due to the absence of intraluminal pressure in the post-mortem state. Importantly, regardless of vein or location within a vein, all valve cusps were similar in architecture, with the opening of the pocket-like leaflet always oriented toward the heart. The main limitation of our study was the inability to evaluate the functional competence of the valves. Also, comparison of our results to prior literature is somewhat limited, since half of our subjects had MS. Previous studies do not make a clear distinction between differing ages and disease types. Nonetheless, post-mortem studies can provide valuable information about the architecture of intraluminal structures. In addition to providing a more thorough understanding of the extracranial venous system, we hope that our discussion and technical approach to dissection will help with future gross anatomical inspection of CCSVI.

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